HEPATIC EFFECTS OF PROPOFOL IN THE HYPOXIC RAT MODEL

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SUMMARY

The aim of this study was to demonstrate the effects of propofol on liver parenchyma in the presence of hypoxic conditions of different levels for different durations of time. 64 Sprague-Dawley rats of both sexes were used in the experiment. They were divided into two groups each containing 32 subjects. In one group rats were given propofol 2 mg/kg intraperitoneally, rats in the other group had no such administration. These two groups were divided into four subgroups (n=8). The subgroups were subjected to mild hypoxia (14% 02) and severe hypoxia (10% 02) for both short term (15 minutes) and a longer term (30 minutes). At the end of the experiment after decapitation, hepatectomy was performed and the specimen sections were examined using a light microscope. The histopathological examination of specimens from the liver of the subjects exposed to severe hypoxia for 30 minutes with propofol showed a significant decrease of congestion, disappearence of vacuolization and an increase in granularity when compared with the specimens of the control group under similar conditions without propofol. However observations of the specimens from the short term experiments either with or without propofol administration did not show such observations. On the other hand in the mild hypoxic group, the specimens from the subgroup treated with propofol showed insignificant granulation and congestion when compared to those without propofol treatment. It is thus concluded that, propofol may protect the liver from the effects of hypoxia by decreasing the oxygen demand of the organ.

Key words: Anesthetics, intravenous: propofol, Liver: hepatotoxicity, Toxicity: hepatic, Hypoxia: liver.

INTRODUCTION

Hypoxia and anesthetic agent toxicity are common clinical problems of the perianesthetic period. Almost all drugs that have been used as anesthetic agents have some side effects. In particular the variable degree of liver damage that may be seen following the administration of fluorinated anesthetic agents being one of the most unpleasant features.

Regarding the aetiology of hepatic injury, hypoxia is known to be a primary cause along with many other predisposing factors. General anesthesia and surgery may interfere with the blood supply and/or oxygen demand of vital organs which are the principle factors in the maintenance of their vitality and functions (1-12).

Due to this point of view, some of the anesthetic agents have been withdrawn from the anesthetic routine. However on the other hand, some brand new agents have become the drug of choice because of their promising effects on blood supply and oxygen consumption of vital organs (13-16).

The intervention of hepatocyte oxygenation by anesthetic agents, especially in hypoxic conditions, have been demonstrated in some studies in hypoxic rat models (8, 9, 17-19).

The aim of this study is to demonstrate the effects of propofol on liver parenchyma in the presence of hypoxic conditions of different levels for different durations.

MATERIALS AND METHOD

64 Sprague-Dawley rats of both sexes varying in body weight from 87 to 304 gr were used in the experiments. The weight and ventilation rate of each rat was measured and recorded before the experimental procedure and then induction of anesthesia was performed using ether inhalation. The duration of the inhalation period was determined according to the clinical signs and the weight of each animal. After positioning the rat, tracheostomy and endotracheal cannulation was performed using a 14 G angiocath. Then the endotracheal cannula was fixed. During these procedures care was taken for maintenance of a satisfactory spontaneous ventilation in each subject. Rats were ventilated with a fresh gas mixture from the flowmeter of Boyle anesthetic apparatus using a Basile

Animal Respirator. Then the spontaneous ventilation was depressed using 70 microgram/kg pancuronium bromide intraperitoneally. Tidal volume of each subject was calculated according to their ventilation rate and weight. Before inhalation of the hypoxic gas mixture rats were subjected to 100% oxygen for three minutes.

The 02/N20 ratios used to produce the mild and the severe hypoxic models were similar to that of Matsumoto (8), Plummer (11) and Lunam (20).

The experiments were performed mainly in two groups, each containing 32 subjects. In one group, rats were given propofol 2 mg/kg intraperitoneally and the subjects in the control group were not. These two main groups were divided into four groups (n=8) according to the intensity and the duration of hypoxia. The subgroups were subjected to mild hypoxia (86% N20-14% 02) and severe hypoxia (90% N20-10% 02) for both short term (15 minutes) and a longer term (30 minutes).

Animals were clinically observed during the experiment. At the end of the experiment after decapitation, laparotomy was performed. Following hepatectomy the liver was taken into a 10% solution of formalin. Removed liver specimens were macroscopically observed before taking a sample from the right lobe. Additionally the section surface of the specimen was examined by naked eye. After routine tissue processing, by using the wider surface of the liver specimens sections were taken and stained with hematoxylineosin. All of them were observed by the same pathologist using the light microscope.

The presence of granules, congestion, vacuolization and Kupffer cells were observed and scored semiquantitavely between 1 to 3. The histopathological data of each subject from each group was compared with that extracted from the other subjects of the same group. In addition, the data of each group was compared with other groups.

Statistical analysis of the observed data was evaluated by a nonparametric test i.e., Wilcoxon Rank Sum Test and the statistic software was Microsoft, 1984 by Ecosoft Inc., and hardware as IBM/AT respectively.

RESULTS

The histopathological evaluation results of the groups in this study are shown in tables I, II, III, and IV.

A liver from the group subjected to a severe hypoxic gas mixture for short term showed a pale appearance only in one subject. In the severe hypoxic group subjected to short term with propofol the section of a liver macroscopically showed a hyperemic appearance with centrilobular necrosis (Table I).

On gross examination of the liver of long term mild or severe hypoxic groups without propofol, hyperemia was the eye-catching feature. On the other hand, in the similar groups with propofol only a pale appearance was noticeable (Tables II, III).

In the group in which the rats were exposed to a mild hypoxic gas mixture for a short term without propofol, the liver showed a pale looking appearance. But, the similar group with propofol showed usually prominent congestion (Table IV).

During microscopic examination of the specimens from the rats exposed to severe hypoxic gas mixture for short term without propofol, granular appearance was noted in almost all cells (2.75±0.46) (Table I). On the other hand, similar groups with propofol did not reflect a significant granulation (0.87±0.64). The difference between the two groups being statistically significant ($p<0.001$).

In the specimens from the animals subjected to long term severe hypoxia with propofol significant intracellular granulation was noted (1.25±1.03) (Figures 1, 2). But in the similar group without propofol granulation was scanty (0.55±0.88). The difference being statistically significant ($p<0.001$) (Table III).

In the specimens from the rats exposed to severe hypoxic gas mixture the presence of congestion was significantly different in comparison with the treatment with or without propofol (values respectively, 0.12±0.35 and 0.55±0.72; $p<0.001$) (Table III, Figure 3).

Additionally, the intracellular vacuolization showed a significant difference in similar groups of long term severe hypoxia with or without propofol (values respectively, 4.50±1.41 and 5.00±2.57; $p<0.001$) (Table III, Figures 4, 5).

Histopathological evaluation of the short term mild hypoxic group accounting granulation showed a significant decrease between two groups in the presence of propofol during the procedures (values respectively, 0.37±0.51 and 1.37±0.51; $p<0.001$) (Table IV). From the point of vacuolization there was also a significant difference between these two groups ($p<0.001$).

Additionally, in specimens from the rats subjected to a long term mild hypoxia the intracellular granulation showed significant difference due to exertion of the group with or without propofol (values respectively 1.00±0.92 and 1.75±1.03; $p<0.001$) (Table II).

DISCUSSION

Since propofol is relatively a brand new agent in anesthetic routine we discovered only a few articles

about the effects of this agent on the liver. Patterson (21) and Kawar (22) showed that propofol has no effect on hepatic function tests. In their original study on cirrhotic patients by Servin (23), it was suggested that mild liver damage triggers no modifications on the pharmacodynamics of the drug.

Based upon a number of previous studies, it is reported that anesthetic agents intensify a preexisting liver damage (3, 8, 9, 11, 12, 17, 18, 19, 24, 25). It is also demonstrated that anesthetic agents plus low oxygen concentration (10%) causes hepatic injury (26-28). This property of anesthetic agents is explained by them causing an increase in the hepatic hypoxia by their depressant effect on the ventilation response of the organism to hypoxia even in subanesthetic doses. Great care is taken to minimize the factors that may artificially affect the hepatic integrity and the blood flow. From this point of view, tidal volume and minute ventilation is calculated precisely according to the weight of each subject, each group being exposed to a gas mixture of equal concentrations. Additionally, in order to eliminate a preexisting hepatic disease great care is taken to choose healthy subjects. Also during dissection and collection of specimens a strict methodology is followed.

In this study the histopathological examination of specimens from the liver of subjects exposed to severe hypoxia for 30 minutes with propofol showed a significant decrease in congestion, disappearance of vacuolization and a significant increase in granulation, indicating cellular defense, when compared with the control group. Data extracted from these results reflects that propofol tends to decrease the hepatic injury caused by hypoxia. The most reasonable explanation of the mechanism of this encouraging effect of the drug may be due to its decreasing effect on the oxygen consumption of the liver. Therefore, it may decrease the oxygen demand of the organ. In studies about the effects of the agent on cerebral hemodynamics, it is noticed that decreased blood flow is accompanied by decrease in oxygen consumption (29-32). We envisage that a similar mechanism may play a role on the hemodynamics of the liver. Mather et al. (33) in their study on sheep, following intravenous propofol administration have demonstrated by varying decreases, i.e. 5%, 15% and 40%, in cardiac output, liver blood flow and total oxygen consumption reflected an absolute decrease in oxygen demand of this organ. From this point of view this particular study of Mather, seems to confirm our hypothesis.

However the histopathological observations of the specimens from the short term experiments of this study with or without propofol did not give appropriate results to verify this theory. The adaptive response of the cells characterized by a significant increase in intracellular granules was prominent and there was no significant vacuolization in the specimens from the rats exposed to severe hypoxia for short term without propofol. We could not conclude whether these histopathological changes were due to an incomplete response to such a short term of hypoxia. Further, it is not clear whether such a short term is enough for propofol to induce some effects on the parenchyma or not.

Whilst studying the specimens from the mild hypoxic group granule formation was not observed. Anyhow the specimens from subgroups which were treated with propofol showed lesser granulation. These results suggested that the rats of the latter group are subjected to the unfavorable effects of hypoxia more than the former one.

Lack of granule formation in these groups of mild hypoxia may be explained by a deficient level of hypoxia. Shingu et al. (12) have demonstrated that even a minute change in the oxygen concentration of gas mixture in hypoxic levels may affect the probability of central lobular necrosis seen by the administration of an anesthetic agent. For instance, by the inhalation of an hypoxic gas mixture with an oxygen concentration of 12-14%, only halothane can cause hepatic injury when compared with enflurane and isoflurane. On the other hand when the oxygen concentration of the gas mixture is decreased to a level of 10% all of these agents may cause similar liver damage.

By evaluating the overall results of our study we deducted that propofol may protect the liver from the destructive effects of hypoxia when adequate hypoxic levels are achieved by appropriate gas mixtures with a proper concentration of oxygen, if the duration is long enough to get the effects of the agent. We assume that, propofol lessens the hepatic injury in hypoxic conditions by decreasing the oxygen demand of the organ. However these results must be confirmed by further ultrastructural studies. Specifically the quantitative and qualitative observations must be performed on the mitochondriae or on probable changes in the intracellular membranes and other organelles. Further we suggest to extend the hypoxic period that may encourage more significant histopathological implications of injury or protection in forthcoming research.

We believe that extension of methods in further studies as mentioned above will mount to our conclusion and provide a more tried and true consensus on this subject.

TABLE I. The histopathological effects of severe hypoxia for short term

PROPOFOL UNTREATED RATS (n=8)				PROPOFOL TREATED RATS (n=8)			
Macroscopic evaluation	Granularity	Congestion	Vacuolization	Macroscopic evaluation	Granularity	Congestion	Vacuolization
Hyperemic	+++	++	-	Pallor	-	-	-
Hyperemic	+++	+	-	Hyperemic *	+	++	+
Hyperemic	+++	++	-	Pallor	-	++	+
Hyperemic	+++	++	-	Pallor	+	++	+
Hyperemic	+++	-	-	Pallor	+	++	-
Hyperemic	+++	++	-	Pallor	+	-	+
Pallor	++	+	-	Pallor	+	+	-
Hyperemic	++	++	-	Pallor	++	+	-
	2.75±0.46**	1.50±0.75			0.87±0.64**	1.11±0.92	5.00±2.25

* Centrlobular necrosis
** $p<0.001$

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TABLE II. The histopathological effects of mild hypoxia for long term

PROPOFOL UNTREATED RATS (n=8)				PROPOFOL TREATED RATS (n=8)			
Macroscopic evaluation	Granularity	Congestion	Vacuolization	Macroscopic evaluation	Granularity	Congestion	Vacuolization
Hyperemic	++	-	-	Pallor	-	+	-
Hyperemic	++	-	-	Pallor	++	-	-
Pallor	+++	-	-	Pallor	+	-	-
Hyperemic	+	+	-	Hyperemic	-	+	-
Hyperemic	+++	+	-	Pallor	++	+	-
Hyperemic	++	+	-	Pallor	-	-	-
Hyperemic	+	+	-	Pallor	++	-	-
Pallor	-	+	-	Pallor	+	-	-
	1.75±1.03*	0.62±0.51**			1.00±0.92*	0.37±0.51**	

* p<0.001
** p<0.001

TABLE III. The histopathological effects of severe hypoxia for long term

PROPOFOL UNTREATED RATS (n=8)				PROPOFOL TREATED RATS (n=8)			
Macroscopic evaluation	Granularity	Congestion	Vacuolization	Macroscopic evaluation	Granularity	Congestion	Vacuolization
Pallor	-	++	+++	Pallor	++	-	-
Pallor	+	-	-	Hyperemic	+	+	+
Pallor	+	-	+	Pallor	+	-	-
Hyperemic	-	-	-	Pallor	-	-	-
Hyperemic	+	+	-	Pallor	-	-	-
Pallor	+	-	++	Pallor	+	-	-
Hyperemic	-	+	++	Pallor	+	-	-
Hyperemic	+	+	-	Pallor	+++	-	-
	0.55±0.88*	0.55±0.72**	5.00±2.57***		1.25±1.03*	0.12±0.35**	4.50±1.41***

* p<0.001
** p<0.001
*** p<0.001

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TABLE IV. The histopathological effects of mild hypoxia for short term

PROPOFOL UNTREATED RATS (n=8)				PROPOFOL TREATED RATS (n=8)			
Macroscopic evaluation	Granularity	Congestion	Vacuolization	Macroscopic evaluation	Granularity	Congestion	Vacuolization
Pallor	+	-	+	Hyperemic	-	+	-
Pallor	+	+	-	Hyperemic	+	++	-
Pallor	+	+	-	Pallor	+	+	-
Pallor	++	-	-	Hyperemic	-	++	-
Pallor	+	+	+	Hyperemic	-	++	-
Pallor	++	++	-	Pallor	-	+	-
Pallor	++	+	-	Hyperemic	-	++	-
Pallor	+	++	-	Hyperemic	+	++	-
	1.37±0.51*	1.00±1.11**	4.50±1.85***		0.37±0.51*	1.62±0.51**	0.00***

* p<0.001
** p<0.001
*** p<0.001

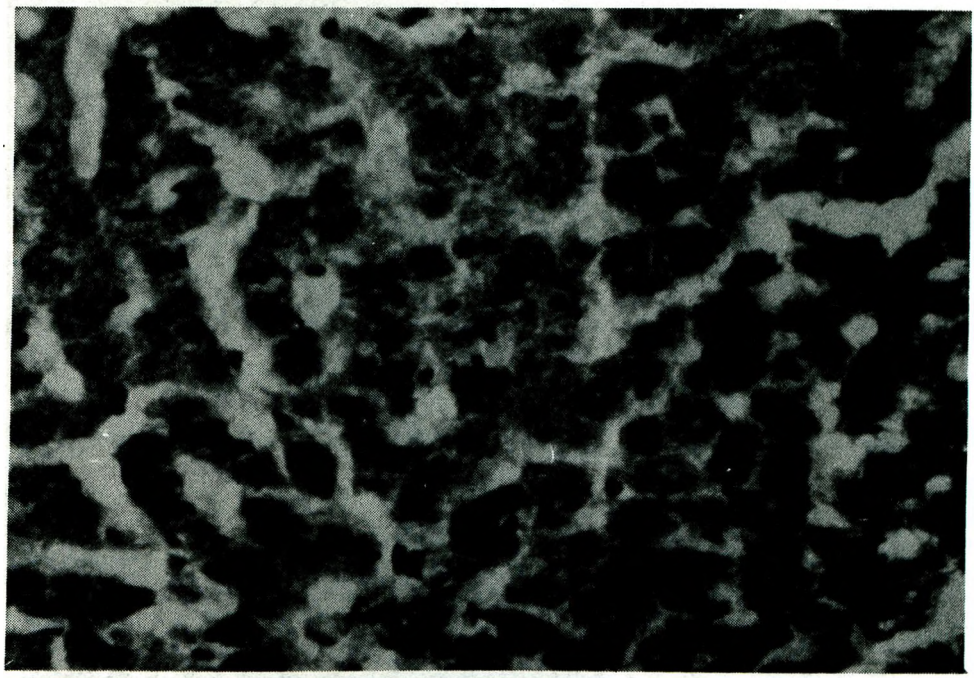

Figure 1. Granular appearance in the hepatocytes (HE; X200)

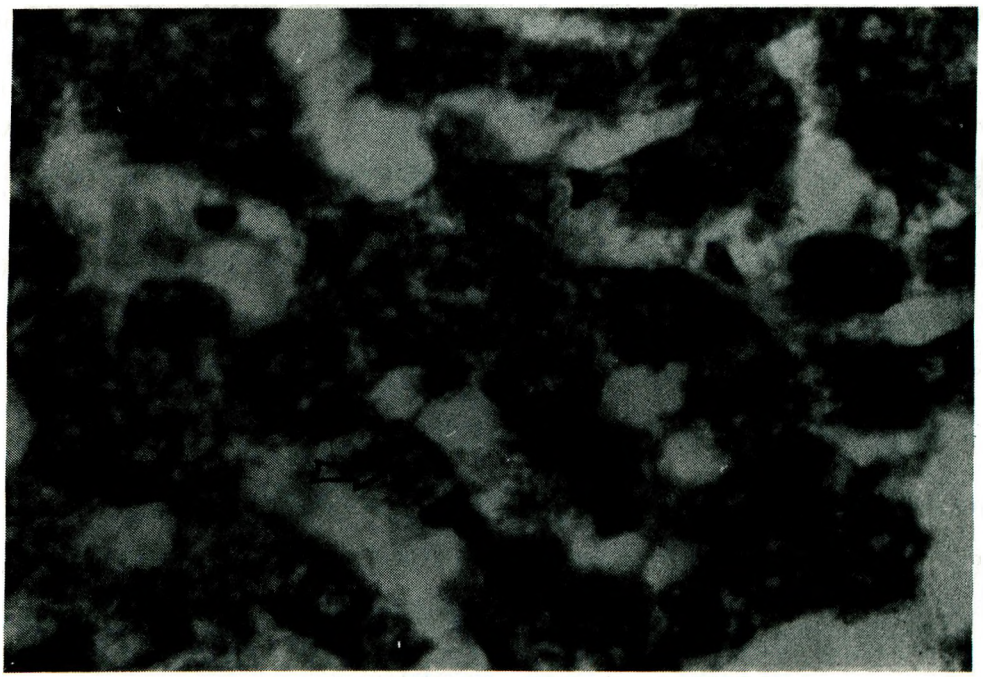

Figure 2. Granular appearance in the hepatocytes (HE; X400)

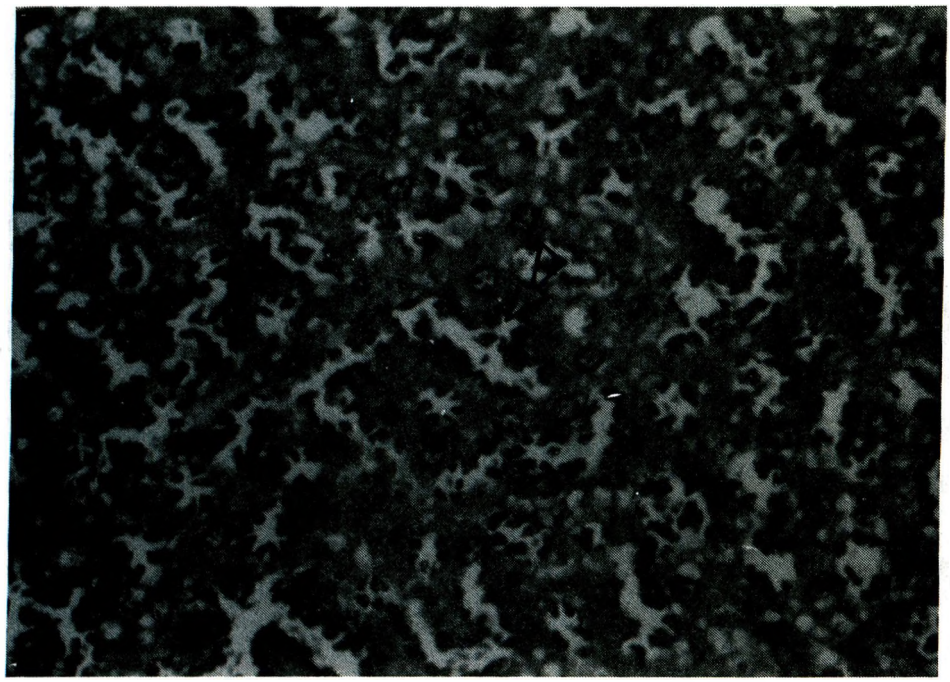

Figure 3. The presence of congestion in the hepatic sinuzoids (HE; X100)

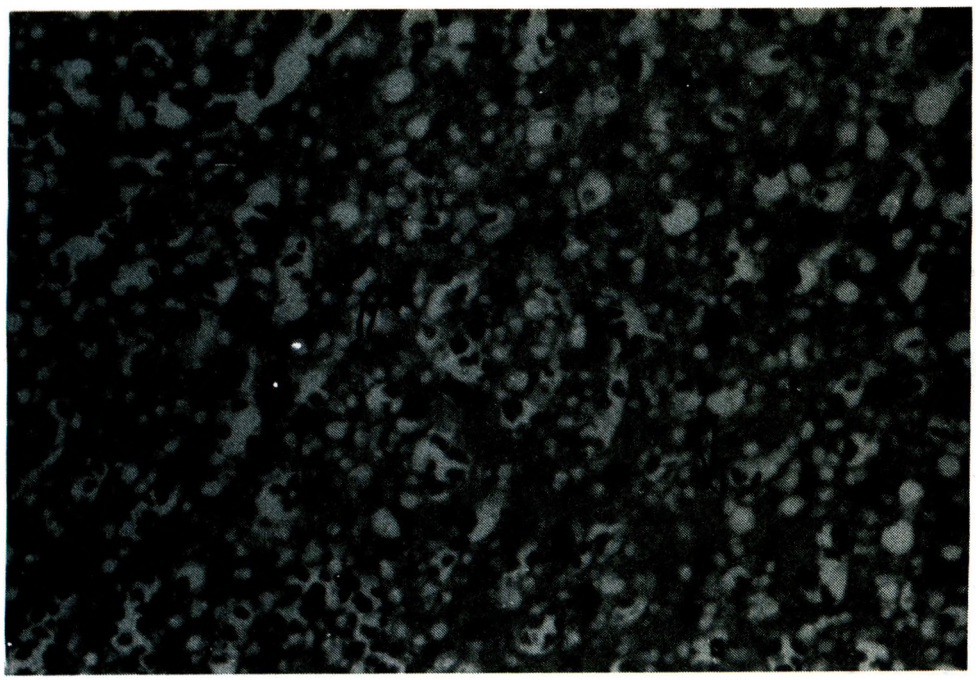

Figure 4. Diffuse mono and multivacuolization in the hepatocytes (HE; X100)

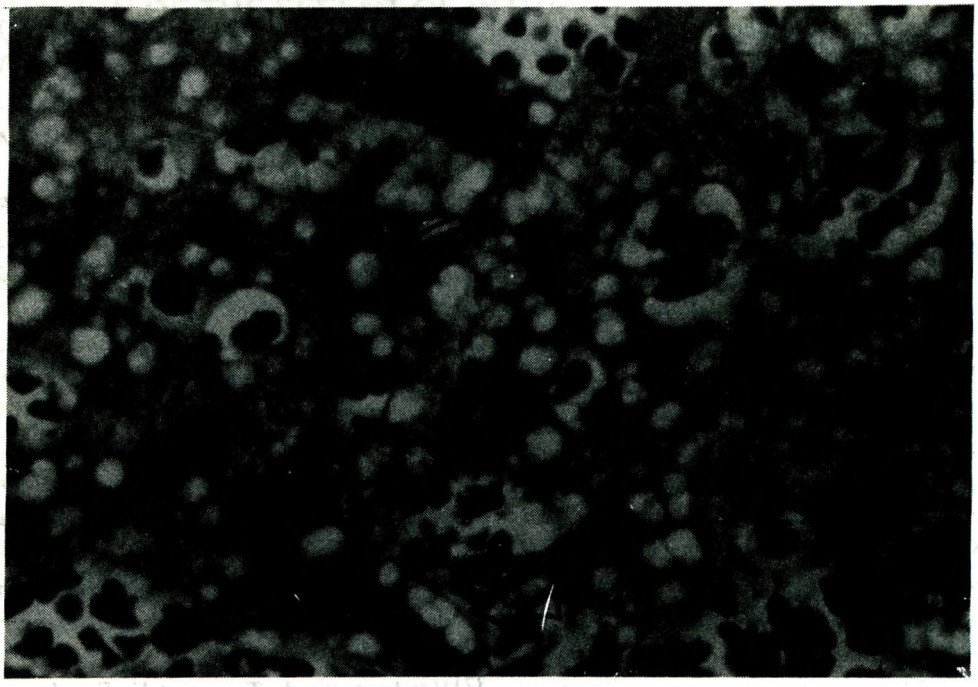

Figure 5. Diffuse mono and multivacuolization in the hepatocytes (HE; X400)

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